



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 058 481
A1

EUROPEAN PATENT APPLICATION

(21) Application number: 82300416.3

(51) Int. Cl.³: A 61 K 37/02
C 08 G 63/08
//C07C103/52, A61K31/765,
A61K9/22

(22) Date of filing: 27.01.82

(30) Priority: 16.02.81 GB 8104734

(71) Applicant: IMPERIAL CHEMICAL INDUSTRIES PLC
Imperial Chemical House Millbank
London SW1P 3JF(GB)

(43) Date of publication of application:
25.08.82 Bulletin 8234

(72) Inventor: Hutchinson, Francis Gowland
29 Woodland Drive
Lymm Cheshire WA13 0BL(GB)

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(74) Representative: Atkinson, John David et al.
Imperial Chemical Industries PLC Legal Department :
Patents Thames House North Millbank
London SW1P 4QG(GB)

(54) Continuous release pharmaceutical compositions.

(57) Pharmaceutical compositions, comprising a polylactide and a pharmacologically active, acid stable polypeptide, which when placed in an aqueous physiological environment release the polypeptide at an approximately constant rate, in an essentially monophasic manner, with a minimal, or no induction period prior to the release; polylactides suitable for use in said compositions; and a method for the manufacture of such polylactides.

EP 0 058 481 A1

TITLE: CONTINUOUS RELEASE PHARMACEUTICAL COMPOSITIONS

This invention relates to pharmaceutical compositions of pharmacologically-active acid-stable polypeptides, which provide continuous release of the polypeptide over an extended period when the composition is placed in an aqueous, physiological-type environment.

It has long been appreciated that the continuous release of certain drugs over an extended period following a single administration could have significant practical advantages in clinical practice, and compositions have already been developed to provide extended release of a number of clinically useful drugs, after oral dosing (see, for example, Remington's Pharmaceutical Sciences, published by Mack Publishing Company, Easton, Pennsylvania, U.S.A., 15th Edition, 1975, pages 1618-1631), after parenteral administration (*ibidem*, pages 1631-1643), and after topical administration (see, for example, United Kingdom Patent Number 1,351,409). A suitable method of parenteral administration is the sub-dermal injection or implantation of a solid body, for example a pellet or a film, containing the drug, and a variety of such implantable devices has been described. In particular, it is known that, for many drugs, suitable implantable devices for providing extended drug release may be obtained by encapsulating the drug in a biodegradable polymer, or by dispersing the drug in a matrix of such a polymer, so that the drug is released as the degradation of the polymer matrix proceeds.

81/08

- 2 -

Suitable biodegradable polymers for use in sustained release formulations are well known, and include polyesters, which gradually become degraded by hydrolysis when placed in an aqueous, physiological-type environment. Particular polyesters which have been used are those derived from hydroxycarboxylic acids, and much prior art has been directed to polymers derived from α -hydroxycarboxylic acids, especially lactic acid in both its racemic and optically active forms, and glycolic acid, and copolymers thereof - see, for example, United States Patents Numbers 3,773,919 and 3,887,699; Jackanicz et al., Contraception, 1973, 8, 227-234; Anderson et al., ibidem, 1976, 11, 375-384; Wise et al., Life Sciences, 1976, 19, 867-874; Woodland et al., Journal of Medicinal Chemistry, 1973, 16, 897-901; Yolles et al., Bulletin of the Parenteral Drug Association, 1976, 30, 306-312; Wise et al., Journal of Pharmacy and Pharmacology, 1978, 30, 686-689 and 1979, 31, 201-204.

In this specification, the term "polylactide" is used in a generic sense to include polymers of lactic acid alone, copolymers of lactic acid and glycolic acid, mixtures of such polymers, mixtures of such copolymers, and mixtures of such polymers and copolymers, the lactic acid being either in racemic or in optically active form. Also, the term "acid-stable" is to be understood as meaning that the polypeptide is not significantly hydrolysed under the conditions encountered within the claimed formulations during the period of use envisaged, that is, at pH 2 at mammalian body temperature, say up to 40°C., for up to six months.

BAD ORIGINAL

81/08

- 3 -

United Kingdom Patent Specification

Number 1,325,209 (equivalent to United States Patent Specification Number 3,773,919) and United States Patent Specification Number 3,887,669 is the only prior art known to us which makes any reference to extended or sustained release of polypeptides, the latter mentioning insulin only, but it contains no specific example of any such formulation, and the reference to polypeptides is apparently entirely speculative, appearing, as it does, only in an extensive listing of very many different classes of drugs which can allegedly be incorporated into formulations of the kind described therein. In fact, essentially all of the other drug types referred to in that specification, apart from polypeptides, are relatively hydrophobic in character and of relatively low molecular weight, and the disclosure of that specification displays no recognition of the difficulties which we have encountered when seeking to obtain satisfactory sustained release formulations of polypeptides, many of which are relatively hydrophilic, and of relatively high molecular weight.

It is to be appreciated that "sustained" or "extended" release of a drug may be either continuous or discontinuous. We have now discovered, in fact, that in many cases when the teaching of the prior art, and in particular the teaching of United Kingdom Specification No. 1,325,209, is applied to the manufacture of a formulation of an acid-stable polypeptide, the release of the polypeptide from the formulation, although occurring over an extended period of time, may also be discontinuous. For example, the release of a polypeptide from a polylactide polymer as described in the said Specification is often preceded

BAD ORIGINAL

81/08

- 4 -

by a significant induction period, during which no polypeptide is released, or is biphasic, and comprises an initial period during which some polypeptide is released, a second period during which little or no 5 polypeptide is released, and a third period during which most of the remainder of the polypeptide is released. By contrast, it is an object of the present invention to provide compositions of acid-stable polypeptides from which, apart possibly from a relatively short 10 initial induction period, the polypeptide is released continuously, with no periods during which little or 15 no polypeptide is released. The words "continuous release" are used in this specification solely to describe a release profile which is essentially monophasic, although it may have a point of inflection, but certainly has no "plateau" phase.

Thus, according to the present invention, there is provided a pharmaceutical composition comprising a polylactide, as hereinbefore defined, 20 and an acid-stable polypeptide, which, when placed in an aqueous physiological-type environment, releases polypeptide into said aqueous physiological-type environment in a continuous manner, as hereinbefore defined, until essentially all of the polypeptide has 25 been released.

This invention is applicable to acid-stable polypeptides quite generally, without any limitation as to structure or molecular weight, but is most useful for polypeptides which are relatively hydrophilic, and 30 the following list, which is not intended to be exhaustive, is indicative of polypeptides which may be employed in the formulation of this invention:-

BAD ORIGINAL

- 5 -

oxytocin, vasopressin, adrenocorticotropic hormone (ACTH), epidermal growth factor (EGF), prolactin, luliberin or luteinizing hormone releasing hormone (LH-RH), insulin, somatostatin, glucagon, 5 interferon, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidin, gramicidines, and synthetic analogues and modifications and 10 pharmacologically-active fragments thereof.

We have found, however, that polypeptides which are not stable under acid conditions, are unsuitable for use in the compositions of this invention, as they become degraded in the acid 15 environment produced in the polymer matrix when the polyactide starts to degrade by hydrolysis, thus producing carboxylic acid end groups.

By "an aqueous physiological-type environment" we mean the body, particularly the musculature or 20 the circulatory system, of a warm-blooded animal, although in laboratory investigations such an environment may be mimicked by aqueous liquids, optionally buffered to a physiological pH, at a temperature of between 35 and 40°C.

The continuous release composition of the 25 invention may be placed in the body of an animal which it is desired to treat with a polypeptide by, for example, intramuscular or subcutaneous injection, or by sub-dermal surgical implantation, in conventional 30 clinical or veterinary manner.

BAD ORIGINAL

- 6 -

We have discovered that a pharmaceutical composition according to the invention may be designed to provide continuous release of the polypeptide by the appropriate choice or control of various parameters, for example by varying the polylactide composition, particularly the proportion of lactic acid to glycolic acid in copolymers; by controlling the molecular weight of the polylactide, both the weight-average molecular weight, and the molecular weight range or polydispersity, measured by the ratio of the weight-average molecular weight, (M_w), to the number-average molecular weight (M_n), i.e. $\frac{M_w}{M_n}$; by choice of the proportion of polypeptide to polylactide; or by choice of the geometry of a solid formulation for implantation, or of the particle size in a formulation for injection. The release characteristics of such compositions are also controlled to some extent by the nature of the polypeptide itself. In particular, there is less freedom of choice in defining the parameters mentioned above when designing a composition of the invention for a polypeptide of high molecular weight, (say, greater than 6000), than there is when designing a composition for a polypeptide of lower molecular weight, (say, less than 6000).

We have further discovered that the release of a polypeptide from a composition comprising a polylactide and a polypeptide proceeds by two distinct and independent mechanisms, namely first a diffusion-dependent release of polypeptide out of the polylactide-polypeptide matrix comprising leaching from the surface, and, for low molecular weight polypeptides, some

81/08

- 7 -

partition-dependent diffusion of polypeptide per se; and subsequently, as the polylactide becomes degraded, diffusion of aqueous polypeptide solution out of the composition through aqueous channels.

In general, the compatibility of polypeptides in polylactide polymers is limited, except in the case of low molecular weight (up to, say, 6000 molecular weight) polypeptides which are capable of having some specific interaction with the polylactide, for example a low molecular weight polypeptide which is basic, and which therefore interacts with the terminal carboxylic acid groups in the polylactide. Because of this limited compatibility of polypeptide in polylactide, a polypeptide/polylactide formulation, when placed in an aqueous environment, releases very little polypeptide by diffusion through the polymer matrix. While this is broadly true for all combinations of polypeptide and polylactide, matrix diffusion is at a minimum for high molecular weight polypeptides in high molecular weight polylactides. Even when some matrix diffusion resulting in polypeptide release occurs initially on placing the composition in an aqueous environment, by release from or very near the surface, this soon ceases because the diffusion of polypeptide into polylactide is insufficient to result in continuous transport of polypeptide from inside the composition to its surface.

When a polypeptide/polylactide composition is placed in an aqueous environment, water diffuses into the matrix, and is partitioned between polypeptide and polylactide to form domains of aqueous polypeptide solution. This aqueous polypeptide, obtained when absorbed water is partitioned between polypeptide and

BAD ORIGINAL

81/08

- 8 -

polylactide, is incompatible with, and insoluble in, polylactides, particularly those of high molecular weight, so that absorption of water by the composition still further reduces initially any likelihood of matrix diffusion of polypeptide out of the composition. If the aqueous domains of polypeptide so formed are discrete and isolated, then the compositions are incapable of releasing polypeptide. However, the possibility of the aqueous polypeptide domains having some continuity increases with increasing concentration of polypeptide in the composition, and with increasing absorption of water, and when the continuity of aqueous polypeptide domains reaches a sufficient level to communicate with the exterior surface of the composition, polypeptide starts to be released from the formulation by diffusion, not through the polylactide matrix, but through aqueous polypeptide channels. Even when some aqueous polypeptide domains near the surface have extended so as to reach the exterior of the composition, that aqueous polypeptide which exists in still isolated domains is not released, and is only released when a secondary hydrophilic path for diffusion becomes available. For high molecular weight polylactides, this secondary hydrophilic diffusion path arises when the polylactide has undergone sufficient degradation for the rate of absorption of water to increase significantly. When this occurs, aqueous pores or channels are generated in the polylactide matrix, which allow a continuous and significant release of polypeptide, as aqueous solution, from previously discrete and isolated domains.

26
O
BAD ORIGINAL

- 9 -

As indicated above, we have discovered that,
when sustained release compositions of the prior art,
and particularly those described in United Kingdom Patent
Specification Number 1,325,209, are used for the release
of polypeptides, the initial matrix diffusion phase of
polypeptide release, and the secondary release of
polypeptide consequent upon degradation of the
polylactide, are separated in time with the result
that the release of polypeptide is not continuous, but
is biphasic and discontinuous, comprising a small first
release of polypeptide, a dead phase during which
essentially no polypeptide is released, and a subsequent
second release phase, during which substantially all
of the remaining polypeptide is released. We have
now discovered that, by appropriate choice of the
parameters of the composition, the matrix diffusion
phase of release, and the subsequent degradation
induced phase of release, can be made to overlap in
time.

Thus, according to a further feature of the invention there is provided a pharmaceutical composition comprising a polylactide, as hereinbefore defined, and an acid-stable polypeptide, and exhibiting two successive phases of release of polypeptide when placed in an aqueous physiological-type environment, the first phase being release by matrix diffusion and the second phase being release consequent upon degradation of the polylactide, characterised in that the diffusion phase and the degradation-induced phase overlap in time.

The two phases can be made to overlap by either extending the initial diffusion phase, or making the degradation-induced phase commence earlier, or both.

81/08

- 10 -

The initial matrix release phase is difficult to extend, but is sensitive to the concentration of the polypeptide in the matrix, and to a limited extent, 30 to the nature of the polypeptide, especially its 5 hydrophilicity,

The degradation-induced release phase can be made to start sooner by appropriate choice of polylactide composition (more glycolide-rich polymer molecules, which degrade more quickly than lactide-rich molecules), 10 M_w (molecules of low molecular weight degrade more quickly to a level at which aqueous channels appear in the matrix), and polypeptide concentration (a higher polypeptide concentration allows more rapid absorption of water, and consequently more rapid generation of 15 continuous aqueous channels which facilitate polypeptide release).

However, as well as requiring the degradation-induced release phase to start earlier, it is also necessary to control the rate of polypeptide release 20 during this phase, and to ensure that the total duration of this phase is sufficient for its intended clinical or veterinary purpose. One method of extending the duration of the degradation-induced release phase, is to use polylactides which contain 25 lactide-rich molecules, which degrade more slowly than glycolide-rich molecules, or alternatively, 15 polylactides containing molecules of high molecular weight, which take longer to degrade to a level at which aqueous channels are formed, can be used.

BAD ORIGINAL

22

- 11 -

15

It is apparent, therefore, that since glycolide-rich polymer molecules, and/or molecules of low molecular weight, are preferred if the degradation phase is to start quickly, and lactide-rich molecules, and/or molecules of high molecular weight are preferred if the degradation-induced release phase is to last for a sufficient period of time, preferred polylactides are those with a high degree of heterogeneity, in respect of glycolide-rich and lactide-rich molecules, or of high polydispersity.

10

Alternatively, the same characteristics can be obtained by blending two or more different polylactides, which differ in lactide/glycolide content, and/or in M_w . In addition, the blending of a minor proportion of polylactide of high M_w with a polylactide of low M_w , confers desirable physical properties upon compositions according to this invention produced therefrom, making them easier to fabricate and process.

15

We have further discovered that the profile of polypeptide release from both prior art polylactides and novel polylactides is almost exactly parallelled by the profile of water absorption. That is to say, when polypeptide release is discontinuous, water absorption is also discontinuous in essentially the same manner, and conversely, when polypeptide release is continuous, so is water absorption. Furthermore, variation of the parameters referred to above for controlling the polypeptide release characteristics of the composition have been found to affect water absorption by the composition in an exactly parallel manner.

20

25

BAD ORIGINAL

- 12 -

Thus, according to a further feature of
the invention there is provided a pharmaceutical
composition, comprising a polylactide as hereinbefore
defined and an acid-stable polypeptide, which when
placed in an aqueous physiological-type environment
absorbs water in a continuous manner, as hereinbefore
defined, until the polylactide has been degraded and
essentially all of the polypeptide has been released
into said aqueous physiological-type environment.

The effect of the various parameters,
referred to above, on the polypeptide release and/or
water absorption characteristics of compositions of
the invention is illustrated by the following experiments:-

A. Molecular weight of the polylactide component.

A.1. Low molecular weight polypeptide.

A.1.1. Formulations were manufactured comprising
20% w/w of the gastric peptide fragment
tetragastrin hydrochloride, Trp-Met-Asp-
Phe-NH₂.HCl, molecular weight = 633, in a
polylactide comprising equimolar proportions
of D,L-lactide and glycolide units, in the
form of a film 0.2mm. thick. The films
were placed individually in water at 37°C.,
which was changed daily, and the ultra-
violet absorption at 277nm. was measured
to assay the tetragastrin released by the
formulation that day.

With a prior art type of polylactide
of M_w approximately 240,000 (intrinsic
viscosity = 1.36), there was an initial
release of tetragastrin, then a "dead
period" from about day 5 to day 21 during

BAD ORIGINAL

- 13 -

which little was released, followed by the main release from day 24 onward.

With a novel polylactide of M_w approximately 15,000 (intrinsic viscosity = 0.25), the release pattern was similar, but the dead period lasted only from day 4 or 5 to day 8 or 9.

With a novel polylactide of low M_w (inherent viscosity of a lg./100ml. solution in chloroform = 0.11), there was no dead period, and tetragastrin was released continuously from time zero (T_0).

A.1.2. Similar formulations were manufactured using 10% by weight of the synthetic luliberin analogue ICI.118,630, \square -Glu-His-Trp-Ser-Tyr-D-Ser(O-tBu)-Leu-Arg-Pro-Azgly-NH₂, molecular weight = 1269, in place of tetragastrin.

With a prior art polylactide of M_w \sim 240,000 (intrinsic viscosity = 1.36), the release of the polypeptide was biphasic, with a dead period of about 15 days.

With a novel polylactide of $M_w \sim 15,000$ (intrinsic viscosity = 0.25), there was a short induction period, followed by continuous release.

With a novel polylactide of low M_w (inherent viscosity of a lg./100ml. solution in chloroform = 0.11), there was continuous release from T_0 .

A.2. Medium molecular weight polypeptide. Formulations were manufactured comprising 0.1% by weight of mouse epidermal growth factor (EGF), molecular weight = 6041, in

BAD ORIGINAL

81/08

- 14 -

5 polylactides comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and of different M_w , and placed in pH 7.4 buffer. Release of EGF was monitored by radio-immunoassay.

10

With a prior art polylactide of $M_w \sim 200,000$ (intrinsic viscosity = 1.08), there was no initial release, and no significant release of polypeptide until between 13 and 20 days after T_0 , after which release was continuous..

15

With a novel polylactide of $M_w \sim 80,000$ (intrinsic viscosity = 0.58), there was also no initial release, and significant release did not occur until between 6 and 10 days after T_0 , after which release was continuous.

20

With a novel polylactide of low M_w (inherent viscosity of a 1g./100ml. solution in chloroform = 0.11), there was continuous release from T_0 .

A.3.

High molecular weight polypeptide.

25

A formulation was manufactured comprising 20% w/w of bovine prolactin, molecular weight $\sim 22,000$, in a novel polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and of low M_w (inherent viscosity of a 1g./100ml. solution in chloroform = 0.11).

30

As expected from previous experiments A.1.1, A.1.2 and A.2, this formulation also released polypeptide continuously from T_0 when tested in vivo in rats, and circulating bovine prolactin assayed by radio-immuno assay.

35

BAD ORIGINAL

81/08

- 15 -

Experiments A.1 to A.3 thus demonstrate that reducing the molecular weight, M_w , or viscosity of the polylactide used in the manufacture of a composition reduces the biphasicity of polypeptide release, for polypeptides of low or medium molecular weight, or the initial delay in release of polypeptides of medium or high molecular weight, and achieves a continuous release of the polypeptide from T_0 .

10 B. Ratio of lactide/glycolide in the polylactide.

Compositions were manufactured in the form of implants containing 100 μ g. (3% w/w) of the luliberin analogue ICI. 118,630 in polylactides of $M_w \sim 300,000$ but of different lactide/glycolide ratios. All of these formulations when tested in vivo in adult female rats exhibiting normal oestrous behaviour, gave biphasic release of the polypeptide, comprising release for about 6 days post-treatment, followed by a dead period, during which there was no significant polypeptide release. The length of this dead period decreased with decreasing lactide/glycolide ratio (L/G), as follows:-

L/G	Dead period (days)
100/0	no release
75/25	51
67/33	34
50/50	15

BAD ORIGINAL

81/08

- 16 -

This experiment thus indicates that a composition exhibiting a biphasic release of polypeptide can be improved in the direction of achieving continuous release by increasing the proportion of glycolide to lactide in the polylactide used, up to about 50% glycolide.

5 C. Ratio of polypeptide to polylactide.

10 Compositions in the form of implants were manufactured using different concentrations of the synthetic luliberin analogue, ICI.118,630, in a prior art 50/50 lactide/glycolide polylactide of $M_w \sim 200,000$, and tested in vivo in rats as described above. At 5% and 10% w/w incorporation, the release of polypeptide was biphasic, but at 15% and 20% incorporation, the biphasicity disappeared.

15 This experiment thus demonstrates that a polylactide of high molecular weight, which gives biphasic release of polypeptide when the polypeptide is incorporated only at low levels, can be used to make satisfactory continuous 20 release formulations if the proportion of polypeptide is increased sufficiently.

25 D. Molecular weight distribution.

15 A solution of a polymer blend of wide molecular weight distribution (polydispersity) was obtained by mixing solutions of a 50/50 D,L- 30 lactide/glycolide polylactide of low M_w , (reduced specific viscosity of a 1g./100ml. solution in chloroform = 0.115), (3 parts by weight) and a 50/50 D,L-lactide/glycolide polylactide of $M_w = 200,000$, (intrinsic viscosity = 1.08), (1 part by weight). Tetragastrin

- 17 -

(1 part by weight) was added, and the mixture was cast to give a polylactide/tetragastrin composition containing 20% by weight of tetragastrin, and this was then moulded to give a slab 0.02cm. thick. The slab was placed in water at 37°C. and the release of tetragastrin was found to be continuous from T_0 , and to continue for at least 44 days.

Polylactides of wide molecular weight distribution may be obtained either by mixing preformed polymers of different molecular weights, or by appropriate control of the polymerisation process in generally known manner, and such polylactides confer important advantages, for example the lower molecular weight polylactide species allow an essentially immediate release of some polypeptide, while the higher molecular weight polylactide species both extend the release period and slow down the overall rate of release of the polypeptide. In addition, the blending of low and high M_w fractions modifies the water absorption characteristics of the polylactide in a parallel manner.

E. Thickness of the implant.

E.1. A solution of 10% by weight of tetragastrin in a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and of $M_w \sim 15000$ was cast into films 0.02, 0.06 and 0.12 cm. thick. All three films showed continuous release of tetragastrin from T_0 , and at 28 days, the three films had released respectively 85, 75 and 66% of their tetragastrin content.

BAD ORIGINAL

- 18 -

10 E.2. The uptake of tritiated water from pH 7.4
2 buffer by slabs of polylactide comprising
3 equimolar proportions of D,L-lactic acid and
4 glycolic acid units of $M_w \sim 200,000$, and
5 intrinsic viscosity = 1.08 and of thickness
0.02, 0.06, 0.12 and 0.20 was measured by
removing such slabs from the buffer solution
successively after varying times of immersion,
and measuring the tritium content by
10 scintillation counting. After 5 weeks, the
different thickness slabs had absorbed
respectively 44, 20, 15 and 11% by weight of
water.

15 These experiments show how the thickness
of the implant can be used to control the
absorption of water by, and thus the rate of
release of polypeptide from, a composition of
the invention, thicker implants releasing the
polypeptide more slowly than thinner ones.

20 As indicated above the composition of the
invention may be formulated as a solid composition for
sub-dermal injection or implantation or as a liquid
formulation for intramuscular or subcutaneous injection.

25 Suitable solid compositions for sub-dermal
injection or implantation are, for example, rods, spheres,
films or pellets, and cylindrical rods which can be
30 injected through a hypodermic needle or trochar are
preferred. Such compositions are manufactured by
conventional techniques which are well known in the
pharmaceutical art.

BAD ORIGINAL

- 19 -

Preferred solid compositions of the invention, for polypeptides within different molecular weight ranges, are as shown in Table 1, and preferred compositions for specific polypeptides of particular interest are as shown in Table 2. Each entry in Tables 1 and 2 thus defines a further feature of this invention.

TABLE 1

No.	Polypeptide M.W.	Polylactide Inherent Viscosity	Glycolide Lactide	% Poly-peptide	Preferred % Poly-peptide
1.	<2000	>0.5	0.5-3	5-50	
2.	<2000	0.2-0.5	0.2-3	5-50	10-30
3.	<2000	<0.2	0-3	0.1-50	
4.	1500-10,000	0.4-0.8	0.5-3	10-50	20-50
5.	1500-10,000	0.15-0.4	0.2-3	5-30	10-30
6.	1500-10,000	<0.15	0-3	0.1-20	1-20
7.	8000-30,000	0.15-0.4	0-3	0.1-50	
8.	8000-30,000	0.1-0.15	0.7-3	10-50	
9.	8000-30,000	<0.1	0-3	0.1-50	

5

6

7

8

BAD ORIGINAL

- 20 -

TABLE 2

No.	Polypeptide	Polyactide Inherent Viscosity	Glycolide Lactide	% Poly- peptide
10.	Tetragastrin	>0.5	1-3	5-50
11.	Tetragastrin	0.2-0.5	0.5-3	5-50
12.	Tetragastrin	<0.2	0-3	0.1-50
13.	ICI.118630	>0.5	0.8-3	5-50
14.	ICI.118630	0.2-0.5	0.2-3	5-50
15.	ICI.118630	<0.2	0-3	0.1-50
16.	EGF	0.4-0.8	0.5-3	10-50
17.	EGF	0.15-0.4	0-3	0.1-50
18.	Prolactin	<0.15	0-3	0.1-50

Also as indicated above, the composition of the invention may also be formulated as a suspension for injection. Such suspensions may be manufactured by general techniques well known in the pharmaceutical art, for example by milling the polyactide/polypeptide mixture in an ultracentrifuge mill fitted with a suitable mesh screen, for example a 120 mesh, and suspending the milled, screened particles in a solvent for injection, for example propylene glycol, water optionally with a conventional viscosity increasing or suspending agent, oils or other known, suitable liquid vehicles for injection.

81/08

- 21 -

The continuous release of ICI.118630 from a suspension composition of the invention was demonstrated by comparing the dioestrous behaviour of normal mature female rats dosed subcutaneously with either a propylene glycol suspension of particles milled to 120-mesh size and containing 3% by weight of ICI.118630 in a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and a $M_w \sim 240,000$, (intrinsic viscosity = 1.36), or with a saline solution of 100, 200 or 300ug. of ICI.118630 per animal. With the saline solutions, there was an immediate short period of dioestrus, but after 3 days post-dosing normal cyclicity was re-established. With the suspension composition of the invention, by contrast, the animals were substantially in dioestrus for about 40 days. Similar results were obtained with an injection formulation based on a mixture of 1% of ICI.118630 in a similar polylactide having an intrinsic viscosity of > 0.5.

Thus, according to a further feature of the invention there is provided a suspension formulation comprising from 1 to 50% by weight of a solid formulation, which itself comprises from 0.1 to 50% by weight of an acid-stable polypeptide as herein defined and from 50 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3 and which is either soluble in benzene and has an inherent viscosity (1g./100ml. solution in benzene) of less than 0.5 or is insoluble in benzene and has an inherent viscosity (1g./100ml. solution in chloroform or dioxan) of less than 4, which solid formulation has been reduced to fine particle size, together with from 50 to 99% by weight of a liquid carrier suitable for injection into mammals.

81/08

- 22 -

It is to be noted that, because of the reduced particle size of the polypeptide/polylactide in a suspension for injection, certain solid formulations, which are not suitable for implantation, are rendered 5 useful when reduced to fine particle size and formulated as a suspension for injection. For example, the two particular suspension formulations referred to above both contain less ICI.118,630 than has been found acceptable for implantable formulations, as shown in 10 Tables 1 and 2 above.

From the foregoing, it is clear that it is desirable to manufacture polylactides of a range of M_w , particularly of low to medium M_w in the range up to 60,000, and of high polydispersity ($\frac{M_w}{M_n}$), these 15 being particularly valuable in the compositions of this invention. The prior art relating to polylactides in general, and to copolymers containing lactic acid and glycolic acid units in particular, is silent as to the manufacture of such copolymers of low molecular weight, 20 and as to methods of achieving high polydispersity in such copolymers. Indeed, it is inherent in the prior art disclosure of polylactides that they are generally of M_w greater than about 30,000-60,000 (an inherent viscosity of 25 greater than 0.5) and of low polydispersity, due to their manufacture under anhydrous conditions and without any added chain-stoppers. We have realised that, because of the different reactivities under polymerisation conditions of the cyclic dimers of lactic acid and glycolic acid, copolymers of high 30 heterogeneity in respect of polymer species may be obtained by ring opening polymerisation of a mixture of the two cyclic dimers in the presence of chain-

BAD ORIGINAL

- 23 -

stopping agents, to give polylactides having an inherent viscosity of less than 0.5. The cyclic dimer of glycolic acid is the more reactive under polymerisation conditions, and thus the first copolymer molecules formed in the polymerisation are glycolic acid-rich. Consequentially, the later copolymer molecules formed are necessarily lactic acid-rich, thus producing a copolymer of lactic acid and glycolic acid of the desired high heterogeneity.

In addition, we control the polymerisation to produce copolymers in the desired low M_w range by carrying out the ring opening copolymerisation of the mixed cyclic dimers in the presence of water, of lactic acid containing water, or of some other known chain growth regulator, in accordance with the general knowledge in the polymer art.

Suitable polymerisation catalysts are zinc oxide, zinc carbonate, basic zinc carbonate, diethyl-zinc, organotin compounds, for example stannous octanoate, tributylaluminium, titanium, magnesium or barium compounds, or litharge, and of these stannous octanoate is preferred.

The copolymerisation of the mixed cyclic dimers is otherwise carried out in conventional manner, known in the polymer art, as regards time and temperature.

Low molecular weight polylactides may also be obtained by copolymerisation of the hydroxy-acids themselves rather than the cyclic dimers. Although less heterogeneous polymers are obtained by this process, suitable matrices for continuous release of

81/08

- 24 -

polypeptides may be obtained by mixing such polylactides of different compositions made by this method, or by mixing a polylactide made by this method with one or more polylactides made by ring opening polymerisation 5 of the cyclic dimers.

Certain of the lactic acid/glycolic acid copolymers described herein are novel and form a further feature of this invention. Thus according to a further feature of the invention there is provided a heterogeneous polylactide comprising from 25 to 100% molar lactic acid units and from 0 to 75% molar of glycolic acid units, and which is either soluble in benzene and has an inherent viscosity (lg./100ml. solution in benzene) of less than 0.5, or is insoluble in benzene and has an inherent viscosity (lg./100ml. solution in chloroform or dioxan) of less than 4. By a "heterogeneous polylactide" we mean polylactides with a high degree of heterogeneity, in respect of glycolide-rich and lactide-rich molecules, or of high polydispersity, or blends of two or more different polylactides which differ in lactide/glycolide content and/or M_w , as hereinbefore described.

Whether or not a particular copolymer is heterogeneous or not, in this sense, may be readily determined from inspection of the 25MHz ^{13}C nuclear magnetic resonance spectrum of the copolymer in, for example, deuterated dimethyl sulphoxide. In a homogeneous copolymer, such as is obtained in the prior art copolymerisation of lactic acid and glycolic acid monomers, the resonance of the glycolic acid unit carbonyl carbon, at $\delta = 166.0 - 166.2$ approximately, appears as two doublets, as a consequence of the four different, approximately equally probable molecular environments in which this carbon atom can exist,

81/08

- 25 -

namely G^*GG , L^*GG , G^*GL and L^*GL (G = a glycolic acid unit, L = a lactic acid unit, and the asterisk indicates the glycolic acid unit under consideration). In a heterogeneous copolymer, on the other hand, such as is used in the present invention, the sequence L^*GL is unlikely to occur, so that one of the doublet signals in the spectrum of the homogeneous copolymer appears as a singlet. In fact, we find that in the spectrum of heterogeneous copolymers, this glycolic acid unit carbonyl carbon signal often appears as two singlets. Thus, a "heterogeneous copolymer" as herein defined is a copolymer for which the glycolic acid carbonyl carbon signal in the ^{13}C n.m.r. appears as other than a pair of doublets.

The heterogeneity or homogeneity of lactic acid/glycolic acid copolymers can also be demonstrated by an examination of their degradation. Thus, when a copolymer is placed in pH 7.4 buffer at $37^\circ\text{C}.$, removed periodically, dried and sampled, and the ratio of lactic acid to glycolic acid units in the samples is determined by n.m.r., for a heterogeneous copolymer, the ratio L/G increases with time, as the glycolic acid sequences hydrolyse preferentially. For a homogeneous copolymer, on the other hand, the ratio of L/G remains essentially constant as degradation progresses.

The lactic acid content of the copolymer is preferably in the racemic (D,L) form, or in the optically active L form.

According to a further feature of the invention there is provided a process for the manufacture of a novel copolymer of lactic acid and glycolic acid as defined immediately above, which comprises the ring opening copolymerisation of a mixture of the cyclic

BAD ORIGINAL

81/08

- 26 -

dimers of lactic acid and glycolic acid, optionally in the presence of a chain growth regulating agent.

A suitable chain growth regulating agent is, for example, water, lactic acid, glycolic acid or other hydroxy acids, alcohols or carboxylic acids generally.

The invention is illustrated but not limited by the following Preparations and Examples:-

Preparation 1

Zinc oxide (16g.) was added to D,L-lactic acid (800g.) in a 2 l. 3-necked round bottom flask-equipped with a stirrer, a thermometer, and a distillation head connected to a water condenser. The mixture was stirred and heated to about 135°C., at which temperature water started to distil over. Heating was continued for 8 hrs., during which time the temperature rose to about 190°C. When distillation of water ceased, the pressure was reduced, and distillation was continued until solid began to collect in the condenser. At this stage the water condenser was replaced by an air condenser, and the residue was cooled and then distilled under high vacuum (2-8mm. of mercury), the fraction (about 300g.) distilling between 130 and 160°C. being collected, this being D,L-lactide (3,6-dimethyl-1,4-dioxan-2,5-dione), the cyclic dimer of D,L-lactic acid.

The crude D,L-lactide was crystallised from ethyl acetate (approximately 600ml.) three times, and the recrystallised product was finally dried at 45°C. under reduced pressure (2mm. of mercury) for 24-48 hours, after which it had m.p. 124-125°C.

BAD ORIGINAL

81/08

30

- 27 -

31

Preparation 2

Glycolide (1,4-dioxan-2,5-dione), the cyclic dimer of glycolic acid, was prepared by the method described in Preparative Methods in Polymer Chemistry by W.R. Sorenson and T.W. Campbell, second edition, published by Interscience (1968), page 363. The crude glycolide was purified by three successive crystallisations from dry ethyl acetate, then dried at 45°C. under reduced pressure (2-8mm. of mercury) for 24-48 hrs., m.p. 82-84°C.

32

Examples 1 to 13

Polymers of D,L-lactide and glycolide were prepared as follows:-

Pure dry D,L-lactide (Preparation 1), pure dry glycolide (Preparation 2) totalling 42g., commercial D,L-lactic acid containing about 12% by weight of water, and 1ml. of an 8% by weight solution of stannous octanoate in hexane, were placed in a pre-dried glass tube. The hexane was evaporated under reduced pressure, and the tube was heated at 160°C. for 6 hours with constant agitation if possible. The tube was cooled in powdered solid carbon dioxide, and the polylactide was removed, broken into small pieces and dissolved in chloroform (400ml.). The chloroform solution was filtered, and the filtrate was poured into methanol (2 l.) to precipitate the polylactide, which was filtered off and dried under vacuum at 40°C. for 24 hours, then at 80°C. for 24 hours. All the polylactides so produced were soluble in chloroform and dioxan, and polylactides 1 to 9 in the following Table were soluble in benzene, but polylactides 10 to 13 were insoluble in benzene.

BAD ORIGINAL

81/08

- 28 -

The following particular polylactides were prepared by this method:-

Ex.	D,L-Lactide (L.) (g.)	Glycolide (G) (g.)	L/G Molar Proportion	D,L-Lactic acid	Intrinsic Viscosity	M_w (approx)
1	42.0	0	100/0	0	1.385	440,000
2	33.5	9.0	75/25	0	1.084	400,000
3	32.4	8.7	75/25	920 μ l.	0.108*	low
4	30.0	12.1	67/33	0	0.97	370,000
5	30.0	12.1	67/33	0	0.94	214,000
6	30.0	12.1	67/33	30 μ l.	0.67	107,000
7	30.0	12.1	67/33	60 μ l.	0.51	63,000
8	30.0	12.1	67/33	120 μ l.	0.37	33,000
9	30.0	12.1	67/33	920 μ l.	0.121*	low
10	23.0	18.5	50/50	0	1.045	300,000
11	23.0	18.5	50/50	400 μ l.	0.25	15,200
12	23.0	18.5	50/50	920 μ l.	0.126*	low
13	23.0	18.5	50/50	1380 μ l.	0.108*	low

M_w are relative to polystyrene standard

* are reduced specific viscosities of a 1g./100ml. solution in chloroform.

Alternatively, the lactide, glycolide and lactic acid if present, may be heated at 160°C. and 0.08g. of stannous octanoate then added to initiate the polymerisation.

BAD ORIGINAL

- 29 -

Example 14

A polylactide comprising equimolar proportions of glycolic acid and D,L-lactic acid units, and having an intrinsic viscosity of 1.36 (50mg.) was dissolved 5 in dioxan (1ml.), and 50 μ l. of a solution of ICI.118630, \square Glu-His-Trp-Ser-Tyr-D-Ser(O-tBu)-Leu-Arg-Pro-Azgly-NH₂ (233mg. per ml. of the acetate salt, equivalent to 200mg. per ml. of base) in distilled water was added. The resultant hazy solution was 10 cast as a film, the solvents were evaporated in a stream of nitrogen in the dark, and the film was dried at 40°C. under reduced pressure (0.02mm. of mercury) for 48 hours. The mixture, containing \sim 17% by weight of ICI.118630 in the polylactide was 15 homogenized by three successive compression mouldings at 110°C. for 10 seconds, and was finally compression moulded into implants 0.038cm. thick, each weighing 1.5mg. and containing 309 \pm 7 μ g. (\sim 17% by weight) of ICI.118630,

20 The continuous release of ICI.118630 from such implants was demonstrated by placing them in female rats exhibiting normal oestrous behaviour. After implantation, the rats went into a period of dioestrus, detected by the lack of cornified vaginal 25 smears, lasting from 31 to 40 days, thus indicating that ICI.118630 was being continuously released during that period.

30 The process described above was repeated, using 50 μ l. of ICI.118630 acetate solution (150mg. of pure peptide per ml. of water) and implants were made

BAD ORIGINAL

- 3C -

similarly, weighing 2mg., containing 306 ± 6 µg. of ICI.118630 (13% by weight), and 0.038cm. in thickness. In the rat oestrus test described above, these implants released ICI.118630 continuously over a period of 5 30 to 38 days, as evidence by the period of dioestrus in the rats.

For use in human therapy, implants containing 1 to 100mg. of ICI 118630 (5-50% by weight), weighing 2mg.-lg., and in the form of cylindrical rods suitable 10 for implantation by trochar, were manufactured by the process described above.

Example 15

The process described in Example 14 was repeated, but using polylactides comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and having intrinsic viscosities of 0.33 and 0.25, instead of 1.36, to prepare implants containing 10% by weight of ICI.118630, weighing about 3mg., and 0.08cm. thick.

These implants were placed in female rats (5 per group) which, prior to implantation, exhibited regular oestrous behaviour. The implants prepared from polylactide of intrinsic viscosity 0.33 exhibited an induction period of 5 days followed by a dioestrus 25 period of about 26 days; and the implants prepared from polylactide of intrinsic viscosity 0.25 exhibited an induction period of 3-4 days, followed by a dioestrus period of about 25 days.

Similar implants, but containing 20% by 30 weight of ICI.118630 were prepared in the same way, and these exhibited a similar dioestrus period, but no induction period, in the rat test described above.

BAD ORIGINAL

81/08

- 31 -

For use in human therapy, implants containing 1 to 100mg. of ICI.118630 (5-50% by weight), weighing 2mg.-lg., and in the form of cylindrical rods suitable for implantation by trochar were manufactured similarly.

5 Example 16

The process described in Example 14 was repeated, using a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and having intrinsic viscosity of 1.36, to prepare 10 mixtures of ICI.118630 and polylactide containing 3% and 1% by weight of ICI.118630. The mixture was micronised at room temperature in an ultracentrifuge mill fitted with a 120-mesh screen, and the micronised particles were suspended in propylene glycol for 25 injection at a concentration of 100mg. per ml.

Female rats showing regular oestrous behaviour were injected sub-cutaneously with 0.1ml. of the 3% by weight propylene glycol suspension described above, or with 0.3ml. of the 1% suspension. Both 20 groups were monitored, by examining vaginal smears daily for cornification, and exhibited occasional cornified smears up to 20 to 24 days after dosing, followed by a clear dioestrus period up to days 38 to 42 after dosing, thus demonstrating continuous release 25 of ICI.118630 over that period.

Example 17

Tetragastrin hydrochloride, (Trp-Met-Asp-Phe-NH₂.HCl), (200mg.) was dissolved in a mixture of dioxan (9ml.) and water (1ml.), and to the solution was added 30 a polylactide as described in Example 11 (1.8g.). The

BAD ORIGINAL

81/08

- 32 -

mixture was cast as a film, and the solvents were evaporated in a stream of nitrogen. The resulting film was dried under reduced pressure (0.02mm. of mercury) at 40°C. for 48 hours, then homogenised by three successive compression mouldings at 80°C. for 10 seconds, and moulded to give films of thickness 0.02, 0.06 or 0.12cm., each weighing about 80mg.

The release of tetragastrin was measured by placing a film in distilled water, removing a sample of the distilled water daily, replacing all the remaining distilled water by fresh, and measuring the ultraviolet absorption of the daily samples at 277nm. The following results were obtained, which demonstrated continuous release of tetragastrin from films of all three thicknesses:-

Time (days)	Cumulative % Tetragastrin Released		
	0.02cm.Film	0.06cm.Film	0.12cm.Film
1	9.6	5.6	4.0
4	14.9	10.5	9.0
7	20.3	13.9	11.7
9	25.3	17.7	14.8
11	33.1	22.6	19.4
14	48.6	33.2	28.1
17	61.9	45.9	40.5
21	74.7	59.8	53.2
24	81.8	68.1	60.2
28	85.2	74.9	66.4
31	86.9	77.7	70.9
36	88.5	82.5	77.5
39	89.2	85.1	82.6

BAD ORIGINAL

Example 18

Tetragastrin hydrochloride (40mg.) was dissolved in aqueous dioxan (1:9 by volume), and added to a solution of:

- 5 (a) a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and having a reduced specific viscosity of 0.115 (as a 1g./100ml. solution in chloroform), (120mg.), and
- 10 (b) a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid residues, and having an intrinsic viscosity of 1.08 (40mg.), in dioxan (2ml.). The mixed solutions were cast as a film, as described in Example 14, and moulded as implants weighing about 50mg., and 0.02cm. thick.

The release of tetragastrin from these implants was measured by the procedure described in Example 17, and the following results showing continuous release were obtained:-

Time (days)	Cumulative % Tetragastrin Released
1	0.6
2	1.0
3	1.6
4	2.7
7	8.6
10	17.2
14	29.4
18	43.1
23	56.3
28	64.9
32	71.2
36	79.0
39	83.9
44	90.5

Example 19

A polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units, and having an inherent viscosity of 0.11 as a 1g./100ml. solution in chloroform, (50mg.), was dissolved in dioxan (1ml.) and a solution of mouse epidermal growth factor (EGF, 0.05mg.) in water (0.05ml.) was added. The mixture was cast as a film on polytetrafluoroethylene cloth, and the solvent was removed in a stream of nitrogen in the dark. The film was dried at 60°C. under reduced pressure (0.8mm. of mercury) for 48 hours. The film was then compression moulded at 120°C. for 10 seconds to give implants 0.02cm. thick, weighing 10mg.

of 0,
in in
in m:
.

BAD ORIGINAL

- 35 -

The implants were each placed in a black vial at 37°C. with 1ml. of McIlvain's buffer (pH 7.4), (Documenta Geigy, Scientific Tables, edited by K. Diem and C. Leutner, published by J.R. Geigy SA, Basle, Switzerland, 7th Edition, 1970, page 280), containing 0.02% by weight of sodium azide. The buffer was removed daily, and replaced with fresh, and the EGF released into the buffer by the implant was measured by radio-immuno assay, which demonstrated that release started immediately, and continued for at least 2 weeks releasing 100-200 μ g. per day.

Example 20

A polylactide comprising equimolar proportions of D,L-lactide and glycolide and having a reduced specific viscosity (lg./100ml. solution in chloroform) of 0.11, (400mg.), was dissolved in dioxan (2ml.), and a solution/suspension of bovine prolactin (100mg.) in distilled water (0.5ml.) was added, with vigorous agitation. The mixture was poured onto a polytetra-fluoroethylene cloth, and dried, first in a stream of nitrogen and then under reduced pressure (0.01mm. of mercury) at 40°C. for 24 hours. The heterogeneous mixture thus obtained was homogenised by four successive compression mouldings at 60°C., and then moulded into a slab 0.2cm. thick, from which implants weighing 60mg. were excised.

The implants were placed subcutaneously into adult female rats, which were then periodically bled from the tail, and the prolactin in the blood samples thus obtained was measured by radio-immune assay. A placebo group, receiving no prolactin in

BAD ORIGINAL

the implant, was similarly assayed, and the levels of circulating prolactin compared. The following results were obtained:-

Time	Plasma level of bovine prolactin (ug./ml.)	
	Placebo group	Treatment group
1	0.38	24.7
2	0.45	105.9
6	0.54	7.7
9	0.72	17.8
13	0.52	65.4
16	0.56	89.7
20	0.75	288
23	0.81	142
26	0.84	562
42	1.25	1250

Example 21

The process described in Examples 1-13 was repeated, except that the polylactide was dissolved in dioxan instead of in chloroform, and similar polylactides were obtained.

Examples 22-29

The process described in Examples 1-13 was repeated, except that the polylactide was dissolved in glacial acetic acid, and the glacial acetic acid solution thus obtained was added dropwise to methanol in order

BAD ORIGINAL

81/08

- 37 -

to precipitate the polylactide, which was filtered off and dried under vacuum at 40°C. for 24 hours, then at 80°C. for 24 hours.

The following particular polylactides were
5 prepared by this method:-

Ex No	D,L-lactide (L) (g)	Glycolide (G) (g)	L/G Molar proportion	D,L-Lactic acid	Intrinsic Viscosity	Mw. (approx.)	stannous octanoate
22	33.3	26.7	50/50	360ul	0.234	13,500	93ul
23	11.1	8.9	50/50	120ul	0.243	14,450	31ul
24	11.1	8.9	50/50	120ul	0.265	16,800	31ul
25	111.0	89.0	50/50	1.2ml	0.257	15,850	0.31ml
26	111.0	89.0	50/50	1.2ml	0.239	14,200	0.31ml
27	88.8	71.2	50/50	0.96ml	0.262	16,600	0.25ml
28	22.2	17.8	50/50	1.38ml	0.09*		62ul
29	20.0	-	100/0	0.12ml	0.260	16,200	31ul

* Inherent viscosity as lgm./100ml. solution in chloroform.

Example 30

A polylactide comprising equimolar proportions
10 of glycolic acid and D,L-lactic acid units and having an intrinsic viscosity of 0.25 was dissolved in glacial acetic acid, and the solution was freeze-dried. The freeze dried powder (540.7mg.) and ICI 118630 (142.1mg.) of the acetate salt (equivalent to > 124mg. of base)
15 were dissolved in 6.8ml. of acetic anhydride-free glacial acetic acid and freeze dried for 24hrs.

BAD ORIGINAL

- 38 -

(The glacial acetic acid was refluxed for 2 hrs. with 1% water to remove the acetic anhydride): The freeze dried product was extruded under pressure at 70°C. to a 1mm. diameter rod, from which implants of the required weight were cut. The implants were dissolved in an appropriate solvent, for example acetonitrile, and assayed for drug content and purity. Implants were shown to contain 16.1% w/w pure I18630 base.

Release of I18630 was evaluated by immersing implants weighing approximately 10mg. in McIlvains pH 7.4 buffer at 37°C. ICI I18630 was released continuously for at least 5 weeks.

In a further experiment, implants weighing approximately 390ug., 860ug., 1500ug., 3000ug and 5600ug were implanted subcutaneously in groups of 5 adult, regularly cycling female rats. In the 28 days following implantation, animals were essentially free of oestrus intervals showing that active drug was released continuously over this period.

) Example 31

A solution of ICI I18630 acetate salt was prepared by dissolving 170.8mg. of ICI I18630 acetate in 5ml. of acetic anhydride-free glacial acetic acid. (The glacial acetic acid was fluxed for 2 hrs. with 1% water to remove acetic anhydride). This solution was shown by high pressure liquid chromatography (HPLC), to contain 25.21mg. of ICI I18630 base per ml. 442.5mg. of polylactide (prepared as in Example 25 was dissolved in 4.5ml. of the acetic acid solution, and the resulting solution was freeze dried for 25 hrs. The freeze dried product was extruded under pressure at

BAD ORIGINAL

74°C. to a 1mm. diameter rod, from which implants of the required weight were cut. The implants were dissolved in an appropriate solvent, such as acetonitrile, and the resulting solution was analysed by HPLC. The 5 implants were shown to contain 20% w/w pure 118630 base.

Example 32

A polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units and having a reduced specific viscosity of 0.126 (lg./100ml. solution 10 in chloroform), (240mg.), was dissolved in glacial acetic acid (5ml.) and a solution of tetragastrin hydrochloride (60mg.) dissolved in glacial acetic acid (5ml.) was added with vigorous agitation. The solution was freeze dried for 24 hrs. and the resultant 15 solid was compression moulded at 50°C. for 20 seconds to give implants 0.2cm thick and weighing 35-40mgs.

The above procedure was repeated with the following polymers:-

- (a) a polylactide comprising 67 mole % of D,L-
lactic acid and 33 mole % glycolic acid units and having
a reduced specific viscosity of 0.121 (lg./100ml.
solution in chloroform).

(b) a polylactide comprising 75mole % of D,L-
lactic acid and 25mole % glycolic acid units and
25 having a reduced specific viscosity of 0.108 (lg./100ml.
solution in chloroform).

(c) a polylactide comprising 100% of D,L-lactic
acid and having a reduced specific viscosity of 0.100
(lg./100ml. solution of chloroform).

0058481

81/08

- 40 -

The release of tetragastrin from these implants was measured by the procedure described in Example 17, and the following results were obtained:-

Time (days)	Cumulative % Tetragastrin released			
	50% D,L- lactide	67% D,L- lactide	75% D,L- lactide	100% D,L- lactide
	50% glycolide	33% glycolide	25% glycolide	
1	6.0	1.0	1.7	1.9
3	12.9	2.1	3.2	3.0
7	23.3	7.1	8.0	5.1
9	27.2	12.2	11.6	6.5
11	30.3	20.2	15.8	8.2
15	36.7	40.0	27.0	14.0
17	39.3	45.0	29.7	17.9
21	44.5	51.8	35.1	25.5
24	49.6	55.6	37.9	30.4
28	58.8	59.6	41.1	36.1
31	68.8	62.3	42.8	40.1
35	81.5	67.3	45.0	45.6
39	91.0	74.3	47.6	50.7
42	95.9	81.9	50.6	55.3
46	96.5	89.1	55.5	60.4
49	97.5	93.2	60.0	65.2
53		95.4	64.8	70.0
56		96.3	68.6	73.6
59		97.0	73.1	77.8
63		97.2	77.1	81.5
70			82.7	86.1
74			85.0	87.5
84			90.4	89.5

BAD ORIGINAL

81/08

- 41 -

These results show that continuous release of tetragastrin is obtained using low molecular weight polylactides and that duration of release is determined by the composition of the hydrolytically unstable 5 polyester.

Example 33

A polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units and having an inherent viscosity of 0.126 (as a 1g/100ml. solution 10 in chloroform) (9.5mg.), was dissolved in distilled dioxan (0.25ml.) and a solution of mouse epidermal growth factor (EGF 0.5mg.) in distilled water (0.1ml.) was added. The mixture was cast as a film on polytetrafluoroethylene cloth, and the solvent was removed 15 in a stream of nitrogen, in the dark. The film was dried at 40°C. under reduced pressure (0.01mm. of mercury) for 48 hrs. The film was then compression moulded at 70°C. for 10 seconds to an implant 0.02cm. thick, weighing about 9mgs. A placebo implant was 20 also prepared.

The samples were implanted subcutaneously into carotid cannulated guinea pigs, blood samples taken periodically and the plasma EGF levels were measured by radio-immuno assay.

Raised plasma EGF levels were observed from Day 3 and continued for at least 1 week.

Similar implants were prepared as described above, but using a polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units 30 and having an intrinsic viscosity of 1.06. Compression

BAD ORIGINAL

- 42 -

moulding of this implant was done at 120°C.

Implantation and plasma assays were done as described above, but raised plasma EGF levels were not observed until day 17 after implantation.

5 Example 34

A polylactide comprising equimolar proportions of D,L-lactic acid and glycolic acid units and having an inherent viscosity of 0.093 as a lg./100ml. solution in chloroform (40mg.) was dissolved in anhydrous-free glacial acetic acid (1ml.) and a solution of mouse epidermal growth factor (EGF, 8.15mg.) in a mixture of water (0.5ml.) and anhydride-free glacial acetic acid (3ml.) was added. The solution was freeze-dried for 24 hrs. The resulting powder was then compression moulded at 50°C. to give an implant 2mm.x 2mm.x 10mm. weighing 36.1mg.

The sample was implanted subcutaneously into a cannulated cat, blood samples were taken periodically and the plasma EGF levels were measured 20 by radio-immuno assay.

Raised plasma EGF levels were observed from day 3 and continued for at least 40 days.

Example 35

Implants containing bovine prolactin were 25 prepared as described in Example 20, but using:-

(a) a polylactide (400mg.) comprising equimolar proportions of D,L-lactic acid and glycolic acid units

20 S
10 x

BAD ORIGINAL

- 43 -

and having a reduced specific viscosity (lg./100ml. solution in chloroform) of 0.11, dissolved in 4ml dioxan; and

20 5 (b) a polylactide (400mg.) comprising equimolar proportions of D,L-lactic acid and glycolic acid units and having an intrinsic viscosity of 1.06 dissolved in 4ml. dioxan. This sample was moulded at 110°C.

10 Formulations (a) and (b) were each tested in vivo as described in Example 20. Formulation (a) released significant levels of plasma bovine prolactin from at least as early as day 4 and continued to release for at least 85 days, while formulation (b) released significant levels from at least as early as day 8 for at least 85 days.

BAD ORIGINAL

What we claim is:-

1. A pharmaceutical composition comprising a polylactide, as hereinbefore defined, and an acid-stable polypeptide, which, when placed in an aqueous physiological-type environment, releases polypeptide into said aqueous physiological-type environment in a continuous manner, as hereinbefore defined, until essentially all of the polypeptide has been released.
- 10 2. A pharmaceutical composition comprising a polylactide, as hereinbefore defined, and an acid-stable polypeptide, and exhibiting two successive phases of release of polypeptide when placed in an aqueous physiological-type environment, the first phase being released by matrix diffusion and the second phase being released consequent upon degradation of the polylactide, characterised in that the diffusion phase and the degradation-induced phase overlap in time.
- 15 3. A pharmaceutical composition comprising a polylactide as hereinbefore defined and an acid-stable polypeptide, which, when placed in an aqueous physiological-type environment absorbs water in a continuous manner, as hereinbefore defined, until the polylactide has been degraded and essentially all of the polypeptide has been released into said aqueous physiological-type environment.
- 20 4. A pharmaceutical composition as claimed in claim 1, 2 or 3 wherein the acid-stable polypeptide is oxytocin, vasopressin, adrenocorticotropic hormone (ACTH), epidermal growth factor (EGF), prolactin, luliberin or luteinizing hormone releasing

hormone (LH-RH), insulin, somatostatin, glucagon, interferon, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidin, gramicidines, and synthetic analogues and modifications and pharmacologically active fragments thereof.

5. A pharmaceutical composition as claimed in claim 1, 2 or 3 wherein the polylactide has a high degree of heterogeneity, in respect of glycolide-rich and lactide-rich molecules, or of high polydispersity.

6. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 5 to 50% by weight of a polypeptide of molecular weight less than 2000 and from 50 to 95% by weight of a polylactide wherein the ratio of glycolide to lactide units is from 0.5 to 3, and which has an inherent viscosity of greater than 0.5.

7. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 5 to 50% by weight of a polypeptide of molecular weight less than 2000, and from 50 to 95% by weight of a polylactide wherein the ratio of glycolide to lactide units is from 0.2 to 3, and which has an inherent viscosity of 0.2 to 0.5.

8. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 0.1 to 50% by weight of a polypeptide of molecular weight less than 2000 and from 99.9 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3, and which has an inherent viscosity of less than 0.2.

BAD ORIGINAL

- 46 -

9. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 10 to 50% by weight of a polypeptide of molecular weight 1,500 to 10,000 and from 50 to 90% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.5 to 3, and which has an inherent viscosity of 0.4 to 0.8.

10. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 5 to 30% by weight of a polypeptide of molecular weight 1,500 to 10,000 and from 70 to 95% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.2 to 3, and which has an inherent viscosity of 0.15 to 0.4.

11. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 0.1 to 20% by weight of a polypeptide of molecular weight 1,500 to 10,000 and from 80 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3, and which has an inherent viscosity of less than 0.15.

12. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 0.1 to 50% by weight of a polypeptide of molecular weight 8,000 to 30,000 and from 50 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3, and which has an inherent viscosity of 0.15 to 0.4.

13. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 10 to 50% by weight of a polypeptide of molecular weight 8,000 to 30,000 and from 50 to 90% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.7 to 3, and which has an inherent viscosity of 0.1 to 0.15.

BAD ORIGINAL

- 47 -

14. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 0.1 to 50% by weight of a polypeptide of molecular weight 8,000 to 30,000 and from 50 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3, and which has an inherent viscosity of less than 0.1.

15. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 5 to 50% by weight of ICI.118,630 and from 50 to 95% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.8 to 3, and which has an inherent viscosity of more than 0.5.

16. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 5 to 50% by weight of ICI.118,630 and from 50 to 95% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.2 to 3, and which has an inherent viscosity of 0.2 to 0.5.

17. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 0.1 to 50% by weight of ICI.118,630 and from 50 to 99.9% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0 to 3, and which has an inherent viscosity of less than 0.2.

18. A pharmaceutical composition as claimed in claim 1, 2 or 3 comprising from 10 to 50% by weight of epidermal growth factor or urogastrone and from 50 to 90% by weight of a polylactide wherein the ratio of glycolide to lactide units is 0.5 to 3, and which has an inherent viscosity of 0.4 to 0.8.

81/08

- 48 -

19. A pharmaceutical composition as claimed in
claim 1, 2 or 3 comprising from 0.1 to 50% by weight
of epidermal growth factor or urogastrone and from
50 to 99.9% by weight of a polylactide wherein the
ratio of glycolide to lactide units is 0.3, and which
has an inherent viscosity of 0.15 to 0.4.

20. A pharmaceutical composition in the form of
a suspension for injection comprising from 1 to
50 % by weight of a solid formulation, which itself
comprises from 0.1 to 50% by weight of an acid-stable
polypeptide as herein defined and from 50 to 99.9%
by weight of a polylactide wherein the ratio of
glycolide to lactide units is 0 to 3 and which is either
soluble in benzene and has an inherent viscosity (lg./
100ml. solution in benzene) of less than 0.5 or is
insoluble in benzene and has an inherent viscosity (lg./
100ml. solution in chloroform or dioxan) of less than 4,
which solid formulation has been reduced to fine particle
size, together with from 50 to 99 % by weight of
20 a liquid carrier suitable for injection into mammals.

21. A heterogeneous copolymer of lactic acid and
glycolic acid units comprising from 25 to 100% molar of
lactic acid units and from 0 to 75% molar of glycolic
acid units, and which is either soluble in benzene and
25 has an inherent viscosity (lg./100ml. solution in benzene)
of less than 0.5, or is insoluble in benzene and has an
inherent viscosity (lg./100ml. solution in chloroform or
dioxan) of less than 4.

22. A process for the manufacture of a heterogeneous
30 copolymer as claimed in claim 21 which comprises the ring
opening copolymerisation of a mixture of the cyclic dimers
of lactic acid and glycolic acid.

0058481

81/08

- 49 -

23. A heterogeneous copolymer as claimed in claim
21 whenever prepared by the process claimed in claim 22.



JOHN DAVID ATKINSON

AUTHORISED REPRESENTATIVE
General Authorisation No. 97

JDA/LMS : PH. 31697 : 12 Jan 82

BAD ORIGINAL

0058481

Application number

EP 82 30 0416.3

European Patent
Office

EUROPEAN SEARCH REPORT

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D	<u>US - A - 3 773 919 (BOSWELL et al.)</u>		A 61 K 37/02
X	* examples 3, 6; column 8, lines 6 to 12 *	21-23	C 08 G 63/08 /C 07 C 103/52
Y	* abstract; column 2, line 60, column 8, line 6 to column 11, line 49 *	1-20	A 61 K 31/765 A 61 K 9/22

Y	Chemical Abstracts vol. 88, no. 12, EuMarch 1978	1-20	
	Office Columbus, Ohio, USA		TECHNICAL FIELDS SEARCHED (Int.Cl.)
	T.M.S. CHANG "Biodegradable semipermeable microcapsules containing enzymes, hormones, vaccines, and other biologicals"		A 61 K 9/22
D	page 79044, column 2, abstract		A 61 K 31/765
X	no 79041n		A 61 K 37/02
Y	& J. Bioeng. vol. 1, no. 1, 1976, pages 25 to 32		C 08 G 63/08

D,A	<u>US - A - 3 887 699 (YOLLES)</u>	1-23	
Y	* claim 1; column 5, line 4; column 6, line 15 *		
	---		CATEGORY OF CITED DOCUMENTS
A	<u>US - A - 4 011 312 (REUTER et al.)</u>	21-23	X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons
P,A	<u>EP - A2 - 0 025 698 (ELI LILLY AND CO.)</u>	21-23	&: member of the same patent family, corresponding document
P	* examples 1 to 7 *		
X	---		
X	The present search report has been drawn up for all claims		
Place of search	Date of completion of the search	Examiner	
Berlin	10-05-1982	BREW	

EPO Form 1503.1 - 06.78

BAD ORIGINAL

0058481

European Patent
Office

EUROPEAN SEARCH REPORT

Application number

EP 82 30 0416.3
- page 2 -

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
- A	<p>Chemical Abstracts vol 91, no. 2, 1979 Columbus, Ohio, USA</p> <p>D. WISE et al. "Results on biodegradable cylindrical subdermal implants for fertility control" page 275, column 2, abstract no. 9442g</p> <p>& Midl. Macromol. Monogr., vol. 5 (Polym. Delivery Syst.), 1978, pages 121 to 138</p> <p>--</p>	21-23	
A	<p><u>GB - A - 2 008 135 (ETHICON, INC.)</u></p> <p>* complete document *</p> <p>-----</p>	21-23	TECHNICAL FIELDS SEARCHED (Int. Cl.)